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Genetic diversity assessment of cereal cyst nematode resistant wheat genotypes using different molecular marker systems

Y.I. Erum*, H. Richou*, A. Mujeeb-Kazi** and A.G. Kazi ***

**Guangdong Entomological Institute, 105 Xingang Road West, Guangzhou 510260, China*

***Wheat Wide Crosses & Cytogenetics, National Agricultural Research Centre, Islamabad, Pakistan*

****Atta-ur-Rahman School of Applied Biosciences, National University of Sciences& Technology, Islamabad*

*Corresponding author's e-mail: erum_i@gmail.com

Abstract

Investigations were conducted to estimate the genetic diversity of wheat germplasm. Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSRs) were employed for this purpose. Six cereal cyst nematode (CCN) resistant wheat genotypes viz., TD-1, SD-8006, Marvi-2000, Moomal-2002, Inqilab-91 and Bhittai were found to be genetically diverse on the basis of RAPD analysis using 14 deca-mer primers. Inferences have indicated that the most diverse genotype was Moomal-2002 as compared to the rest of the genotypes studied and the most effective loci to screen diversity was found to be OPA-09. Forty six SSR primers performance expressed that the genotype (Marvi-2000) was the most diverse and it was also identified as the most resistant genotype against CCN. It is therefore recommended to introduce Marvi-2000 in the breeding program for wheat improvement against CCN.

Wheat is attacked by a number of pests and diseases. Among them, nematodes have been considered universally as one of the important microscopic organisms. Cyst nematodes represent a large group of parasites of economically important crops such as wheat, which is one of the most targeted hosts. The development of molecular marker technologies during the last ten years has revolutionized the genetic analysis of crop plants. RFLPs, RAPDs, AFLPs and SSRs have been used for assessing variation in local and global collections of wheat germplasms. RAPD and microsatellite markers have been considered to be the markers of choice for assessment of genetic diversity among wheat cultivars and their wild relatives (Pestsova *et al.*, 2000). RAPD involves the use of a single arbitrary primer in a PCR reaction and results in the amplification of several discrete amplicons. Each product is derived from a region of the genome that contains two short segments in inverted orientation, on opposite strands that are complimentary to the primer and sufficiently close together for the amplification to work. RAPD markers inherited in a dominant manner are not transferable from one population to another and are poorly reproducible between different laboratories (Welsh & McClelland, 1990; Devos *et al*., 1992; Yu & Paul, 1993). Simple sequence repeats (SSRs) are abundant in most genomes and are accessible by PCR where flanking DNA sequence is available. Their ease of use and high information content has ensured that they have largely replaced RFLP as a mapping technology in different organisms. In plants their developments are promoting and well in access in the relativelyless diverse species like wheat (Roder *et al*., 1995) and barley (Liu *et al*., 1996). SSR loci are now being incorporated into the established genetic maps of the entire major creates (Smith *et al*., 1997; Akagi *et al*., 1996; Korzun *et al*., 1997; Stephenson *et al*., 1998). In view of the magnitude of the problems caused by cyst nematodes, there is an absolute need to carry out further studies on the genetic diversity of wheat germplasm in Pakistan.

Materials and Methods

Plant material: Six indigenous bread wheat cultivars (TD-1, SD-8006, Marvi-2000, Moomal-2002, Inqilab-91 and Bhittai) that have already been screened against cereal cyst nematode (CCN) and scored resistant were selected for this study. Varietal description is given in Table 1.

Table 1. Descriptions of the germplasms.

Varieties	Average yield	Adaptability area	Resistance	Breeders	Organization
Bhittai	5500 kg/ha	Whole Sindh	Stem, leaf, stripe rust, Loose smut, Kernal burnt, Lodging and Drought.	Shamadad Khanzada and Imdad Hussain Korejo	Pakistan Atomic Energy Commission
TD-1	5345 kg/ha	Some areas of Sindh	Stem and Stripe rust, Susceptible to yellow, leaf rust.	Karam Khan Kaleri	Agricultural Research Institute, Tandojam
Marvi-2000	5868 kg/ha	Whole Sindh	Stem, leaf, stripe rust, loose smut, kernel burnt and lodging.	Shamadad Khanzada and Imdad Hussain Korejo	Pakistan Atomic Energy Commission
Inqilab-91	4500 kg/ha	Irrigated areas of Punjab	Stem and leaf rust	M.H. Chaudhry, A. Sattar and F.A. Khan	Wheat Research Institute, Faisalabad
SD-8006	5818 kg/ha	Whole Sindh	Leaf and yellow rust.	Shamadad Khanzada and Imdad Hussain Korejo	Nuclear Institute of Agriculture, Tandojam
Moomal-2002	4955 kg/ha	Whole Sindh	Stem and Stripe rust.	S.H. Nankani	Wheat Research Institute, Sukrand

DNA extraction: DNA was extracted according to the CTAB protocol of Saghai *et al*., (1984).

Random Amplified Polymorphic DNA (RAPD) analysis: For RAPD analysis, the concentration of genomic DNA, 10X PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs, 10 mer random primer and Taq DNA polymerase were optimized. The 10-base oligonucleotide primers obtained from Integerated DNA Technologies, Inc., (USA) were used for the amplification of the genomic DNA (Table 2). Taq polymerase buffer, $MgCl_2$ and dNTPs were purchased from Fermentas (Italy). DNA amplification reactions were performed in a thermal cycler (Eppendorf).

The PCR profile followed was: one cycle of 93 ºC for 5 min; 40 cycles of 94 ºC for 1 min; 36 ºC for 1 min; 72 ºC for 2 min and a final extension for 10 min at 72 ºC. The RAPD fragments were analyzed by electrophoresis on 1.2 % agarose gels with ethidium bromide (10 ng/100 mL of agarose solution in Tris borate EDTA buffer). A 1000 bp ladder was used as marker for RAPD primers. Observations were taken under transilluminator at 320 nm wavelength.

Simple Sequence Repeats (SSRs) analysis: Forty-six primer pairs were used to characterize loci containing microsatellite sequences among the 6 bread wheat cultivars/lines (Table 2). Information on primer sequence is available at http://wheat.pw.usda.gov/index.shtml and primers were synthesized by e-oligos, Gene Link, USA.

PCR reactions were performed in 12µl reaction, containing 0.5U Taq polymerase, PCR buffer, 1.5 mM $MgCl_2$, 0.2 mMdNTPs, 0.15µg/µL of primers and 100 ng of template DNA. The PCR cycling profile used was: 94 ºC for 4 min. (1 cycle); 94 ºC for 1 min; 36 ºC for 1 min; 72 ºC for 2 min (35 cycles) and 72 ºC for 10 min. High-resolution metaphor agarose and agarose were dissolved to prepare (2.5 %) gel in TBE buffer by heating for 5-10 minutes in the microwave. Ethidium bromide in the amount of 4 µl was added after a little cooling and then swirling the solution for a second.

Table 2. List of RAPD and SSR primers used.

RAPD ten-mer primers	Sequences (5'-3')
OPA-01	CAGGCCCTTC
OPA-02	TGCCCAGCTG
OPA-04	AATCGGGCTG
OPA-07	GAAACGGGTC
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCCG
OPA-12	TCGGCGATAG
OPA-14	TCTGTGCTGG
OPB-03	CATCCCCCTG
OPB-06	TGCTCTGCCC
OPD-04	TCTGGTGAGG
OPD-11	AGCGCCATTG

SSR primers	Forward primer	Reverse primer
WMC-103	AGCGTTGTCTAGGTTTAGGTTG	TAGTAGCTAGCCAGATGGATT
WMC-104	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA
WMC-105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG
WMC-107	GAATTCAGGCCCTTCTCGGA	CATTGAACCTCGCATAACGG
WMC-108	TCGAAGAGTAGAGTCGCTCATA	ACATACGTGTCTTAAGTGG
WMC-109	AATTCGGGAAGAGTCTCAGGGG	TTCGAAGGGCTCAAGGGATACG
WMC-110	GCAGATGAGTTGAGTTGGATTG	GTACTTGGAAACTGTGTTTGGG
WMC-111	ATTGATGTGTACGATGTGCCTG	CATGTCAATGTCATGATGAAGC
WMC-113	CAAGTTGACCATCGACTCATAA	AACTGGAAGCCACGACAACATA
WMC-114	AACGACGGCCAGTGAATTCCTC	AGCATCGACATGCAACAACCCC
WMC-116	ACGGCACCCCAGAACCTAGTCG	GCGTGCGTCTGTGCTGCATCGG
WMC-117	ATGCTCCTCCTAGAGCATGTGT	TCGAGATGTCGATTCGTTTTCC
WMC-118	AGAATTAGCCCTTGAGTTGGTC	CTCCCATCGCTAAAGATGGTAT
WMC-119	TGTGTGTTGTGAGACGGGATTG	GCCACCTATTGTAGGTGGATGTT
WMC-120	GGAGATGAGAAGGGGGTCAGGA	CCAGGAGACCAGGTTGCAGAAG
WMC-121	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA
WMC-122	CAGAATTTCTCGTAGAACCA	GGCATTGTTGTTAAAGCACT
WMC-123	TGTAGGGGTAGGTCGATGTGTC	GGATGGTAACATGCATGTCTTC
WMC-124	CGGAGGCGGTCGACTGGATT	GTGCCCAGCAGGGACGAGGA
WMC-125	ATACCACCATGCATGTGGAAGT	ACCGCTTGTCATTTCCTTCTGT
WMC-126	TTGTCACAAAAGGGTCGTAAAT	ATCTCGAGATGTCATCGAAAAT
WMC-127	TTGACTAACGGGTTTACCTGGC	GTTGACCTTCTGCTGTCATTGC
WMC-128	CGGACAGCTACTGCTCTCCTTA	CTGTTGCTTGCTCTGCACCCTT
WMC-129	TGAGAGCCGACCTCCTCTTACT	CGGAGACGGTCGACTGGATTAT
WMC-130	TGTGGTGCCAACTTCTTCTATG	CTCCGTGACTTCTCTCTCAAAA
WMC-131	GCAACGTCATGCTCCGATAAC	GCATCCAGTCCTTCTTCGCTT
WMC-132	CTAGGTCACATTGCGGCGAAGT	GACGGCCAGGAGGATTATCAGT
WMC-133	CATCTCGATCTCATCGAGAAGG	ACCGCGTCAACTACTTAAGCCA
WMC-134	CCAAGCTGTCTGACTGCCATAG	AGTATAGACCTCTGGCTCACGG
WMC-135	CAACCATCAACAGCCACACAGC	GCCATATAGCCTTGGATGGGGA
WMC-136	GTATGATGACACATGGTGCTGC	GACCTGATTCTGCTGTGCTCTG
WMC-137	CGAGAAGTCTACATATCGAGGG	CAACAATGACAACAGAAGGGTG
WMC-138	AATCCCGGTCAAATGAGAAT	GAGACATGCATGAATCAGAA
WMC-139	TGTAACTGAGGGCCATGAAT	CATCGACTCACAACTAGGGT
WMC-140	CCCTCTCCTCGGGTGTTGCTTG	CCCAGGAGCCCTCATGCATACG
WMC-141	TGCAAGGGGTTGAGTTCTTAGG	TTGATAGAGCAAGACGAAGGGC
WMC-142	AACCGCAACTCTTGTGACCGTG	GCTAGACCTGCCTGTCGGTATG
WMC-143	CTTGACCCAAGTAGTTCTTTCC	GTGGGTAAAGTGTGCAACCTCT
WMC-144	GGACACCAATCCAACATGAACA	AAGGATAGTTGGGTGGTGCTGA
WMC-145	GGCGGTGGGTTCAAGTCGTCTG	GGACGAGTCGCTGTCCTCCTGG
WMC-146	CCAAGTGGTTCTTTCCATCATC	GTGGGTAAAGTGTGCAACCTCT
WMC-147	AGAACGAAAGAAGCGCGCTGAG	ATGTGTTTCTTATCCTGCGGGC
WMC-148	CCGATGAAGCGTGAGTAGTTTA	GATCTCAACAAACACACTGCCA
WMC-149	ACAGACTTGGTTGGTGCCGAGC	ATGGGCGGGGGTGTAGAGTTTG
WMC-150	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA
WMC-151	TACGGCCTAGAAGATATGAGCA	TTGTTGTTTTGGAACGCTTGTC
WMC-152	CTATTGGCAATCTACCAAACTG	TCTCTTCTTGCCACATATTCGT

The solution was then poured in a gel casting tray with comb inserted for formation of wells. Samples were loaded after adding loading dye.

DNA fragments were separated using 35V for 4 hrs. A 50bp ladder was used as marker for microsatellite primers, observed on UV trans-illuminator at a wavelength of 320 nm.

Data scoring and genetic analysis: The bands were counted by starting from the top of the lanes to the bottom. All visible and unambiguously scoreable fragments amplified by the primers were scored under the heading of total score able fragments. Amplification profiles of the six genotypes were compared with each other and bands of DNA fragments were scored as present or absent. Detailed analysis was done by utilizing Nei and Li's genetic similarity and distance matrix (Nei & Li, 1979).The equation used is: Number of shared amplification products = 2 x (Number of common bands between any two lanes)/(Total number of bands in the same two lanes).

Genetic relationship among the genotypes was estimated with the dendrogram constructed using unweighted pair group of arithmetic means (Sneath & Sokal, 1973) using software PHYLIP version 3.5; effective number of alleles (Hartl & Clark, 1989); Gene diversity between loci and Shannon's Information index (Shannon & Weaver, 1949) has been calculated by using POPGEN 32 version 1.31.

Results

The polymorphism data of wheat genotypes has shown the effectiveness of RAPD and SSR markers (Table 3). In RAPD analysis, 14 decamer primers were used for amplification of all 6 genotypes; all primers gave reproducible and scorable amplicons. This scorable data was further used for population genetics analysis with emphasis on genetic diversity.

A total of 185 bands were generated with an average of 13.21 bands/primer, all of which were polymorphic up to 80.5% for the 6 genotypes. The highest number of polymorphic loci/assay entity was 14 for TD-1 whereas lowest polymorphic loci number was found to be only 9 for SD-8006 (Table 4).

In SSR analysis, a total of 109 fragments were detected using 46 SSR primers. The number of polymorphic band/assay unit ranged from 43 to 46 (average of 44.39 bands/primer). The summary of RAPD and SSR banding patterns has been shown in Fig. 1 (A, B).

Estimation of genetic diversity: The details of the genetic distance were estimated between pairs of genotypes that were calculated for each marker system, shown in Table 5 and 6 for RAPD and SSR, respectively. RAPD data has shown low average distance than SSRs. Estimated distance based on genetic influence across 68 polymorphic loci ranged from 0.0034 to 0.0228 for Marvi-2000/Inqilab-91 and Moomal-2002/Bhittai. The results revealed that for 266 polymorphic SSR bands, the lowest GD (genetic diversity) value was 0.0004 for Moomal-2002/Inqilab-91 and the highest for TD-1/Bhittai i.e., 0.0032. This proves SSR as a very effective tool to reveal polymorphism.

Cluster analysis: Based on the matrix of the genetic similarity values, UPGMA clustering method was used to construct the dendrogram, which finely elucidated the results of RAPD and SSR data profiles. Both dendrograms are entirely different from each other. Genetic distance gained for both marker systems was significantly different for all six genotypes, ranging between 0.1 to 1.0 and 0.02 to 0.1 for RAPD and SSR, respectively, showing that SSR screened the diversity at high-resolution power.

Genotype (Moomal-2002) was highly diverse from the rest of the five genotypes, on the basis of RAPD data GD=1.04 but the same genotype was at 0.1 genetic distance from the genotype TD-1 in the dendrogram constructed from SSR markers. Based on the RAPD markers, the two nearest cultivars were Inqilab-91/Bhittai (GD=0.12), while the 2 distinct individuals were Moomal-2002/Bhittai (GD =1.04).

Table 3. Effectiveness of RAPD and SSR markers in detecting polymorphism of wheat cultivars/lines.

Analysis	RAPD	SSR
Number of assay unit	14	46
Total bands scored	185	654
Polymorphic bands scored	68	266
% of polymorphism	80.95	96.43
Minimum polymorphism scored / genotype	9	97
Maximum polymorphism scored / genotype	14	117
Average polymorphism scored / genotype	11.33	44.33

Table 4. Polymorphic loci data in relation to wheat genotypes.

Genotypes	Total bands		No. of polymorphic loci		% of polymorphic loci	
	RAPD	SSR	RAPD	SSR	RAPD	SSR
TD-1	43	97	14	43	100	93.48
SD-8006	22	116	9	45	64.29	97.83
Marvi-2000	22	104	10	44	71.43	95.65
Moomal-2002	37	115	12	45	85.71	97.83
Inqilab-91	29	117	12	46	85.71	100
Bhittai	32	105	11	43	78.57	93.48

Table 5. Similarity matrix of RAPD data.

Pop ID	1	2	3	4	5	6
1	****					
2	0.0117	****				
3	0.0130	0.0064	****			
4	0.0172	0.0207	0.0220	****		
5	0.0097	0.0057	0.0034	0.0220	****	
6	0.0102	0.0062	0.0051	0.0228	0.0036	****

Nei & Li (1979), Original measures of genetic distance; TD-1 (1), SD-8006 (2), Marvi-2000 (3), Moomal-2002 (4), Inqilab-91 (5), Bhittai (6).

Table 6. Similarity matrix of SSR data.

Pop ID	1	2	3	4	5	6
1	****					
2	0.0023	****				
3	0.0024	0.0018	****			
4	0.0013	0.0028	0.0022	****		
5	0.0015	0.0029	0.0021	0.0004	****	
6	0.0032	0.0017	0.0028	0.0029	0.0030	****

Nei & Li (1979), Original measures of genetic distance; TD-1 (1), SD-8006 (2), Marvi-2000 (3), Moomal-2002 (4), Inqilab-91 (5), Bhittai (6).

A

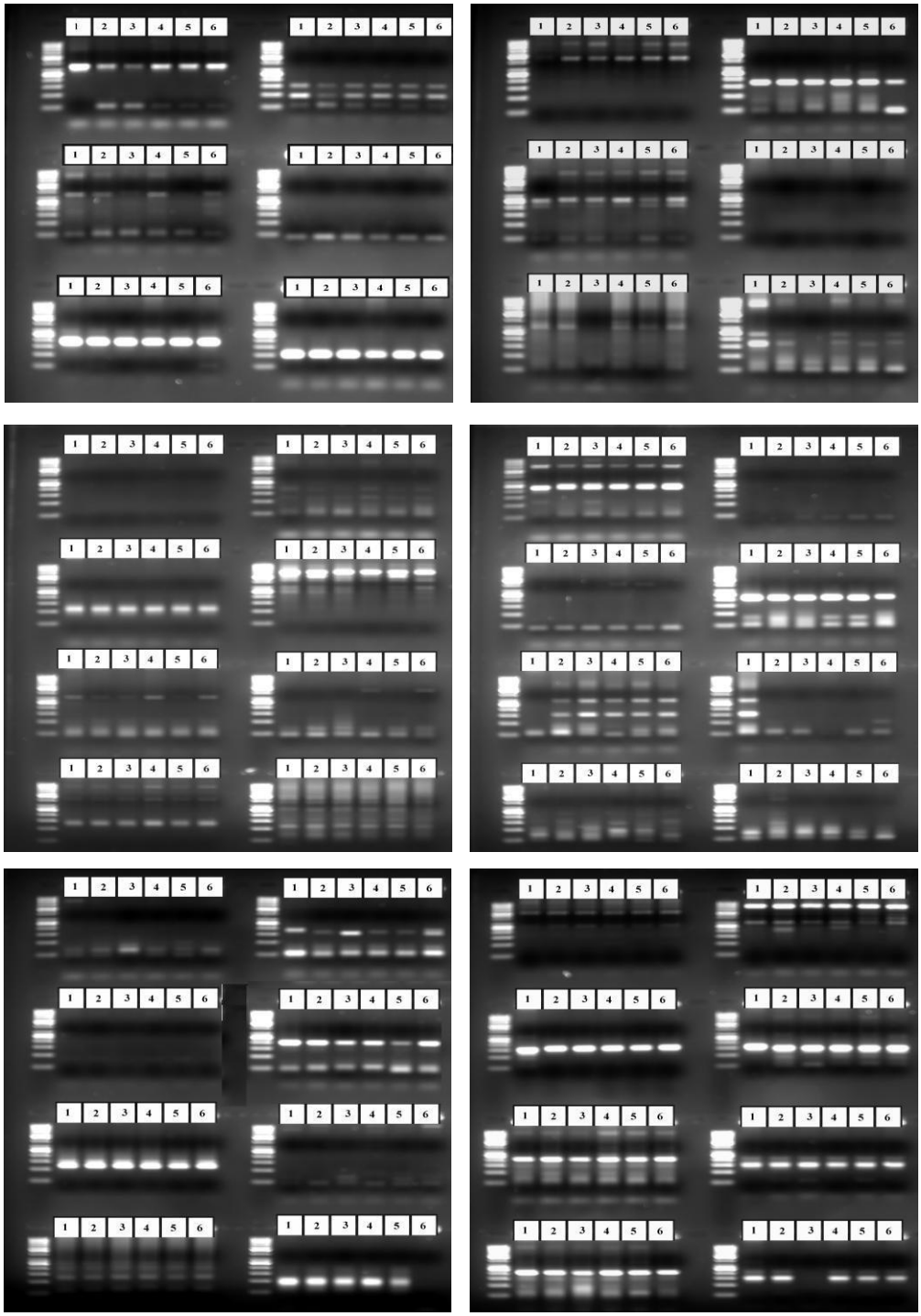

Fig. 1. A. Trans-illuminated gel images of SSR markers; Varieties screened are: 1. TD-1; 2. SD-8006; 3. Marvi-2000; 4. Moomal-2002; 5. Inqilab-91; 6. Bhittai.

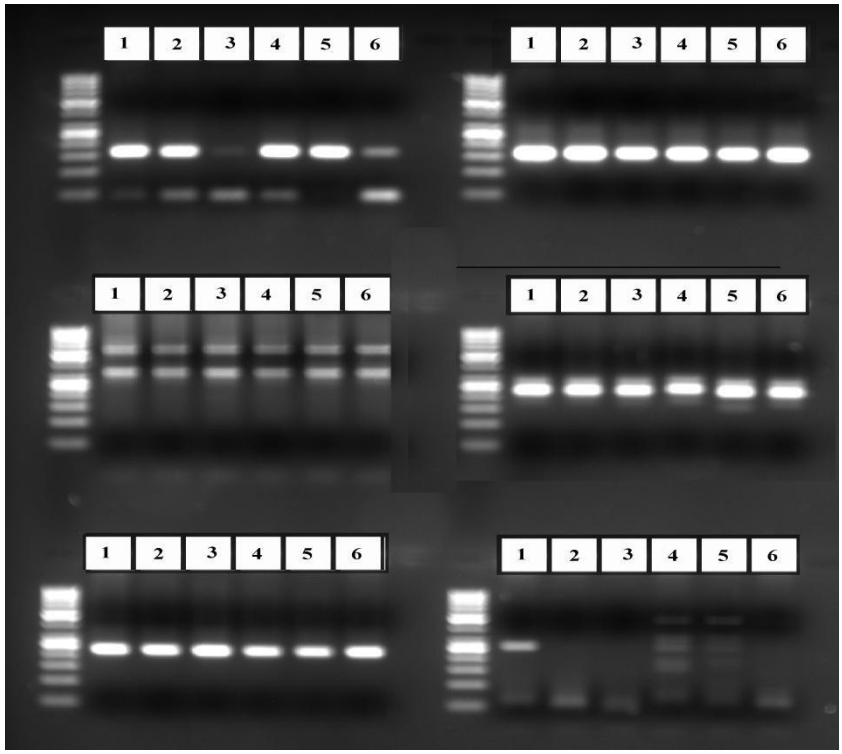

Fig. 1. B. Trans-illuminated gel images of RAPD markers. Varieties screened are: 1. TD-1; 2. SD-8006; 3. Marvi-2000; 4. Moomal-2002; 5. Inqilab-91; 6. Bhittai.

For the SSR markers, GD values varied from 0.08 (between SD-8006/Bhittai) to 0.11 (Marvi-2000 and three of the remaining varieties viz., TD-1, Moomal-2002 and Inqilab-91) showed an average value of 0.02.

Dendrogram interpretation using RAPD and SSR primers: (a) The dendrograms produced for RAPD markers allow defining three groups (Fig. 2 A, B). The first group consists of SD-8006 and Marvi-2000 (GD = 0.2). The SD-8006 genotype is derived from the cross with Marvi-2000 in its parentage (ZARDANA/MARVI-2000/J-78). This parentage explains the high genetic similarity obtained by the RAPD markers. The third individual of this group does not have a pedigree relation with the rest of the 2 genotypes but has GD = 0.5. The second group consists of Inqilab-91 and Bhittai with GD = 0.12. Both have different genetic background but share the common character of high yielding potential under favorable conditions. Moomal-2002 is genetically diversified from all the studied genotypes (GD = 0.1) as it originated by the combination of Irani and indigenous germplasm.

(b) SSR markers have depicted quite different hierarchical classification based on the genetic distance as compared to RAPD.

Three groups are defined again but none has consistency with the RAPD cluster. The fact is, microsatellite markers are good enough to calculate the genetic diversity as they advocated a highly efficient alternative because this offers rapid and precise selection due to their hyper variability and high-resolution power. The first group comprised of three genotypes TD-1, Moomal-2002 and Inqilab-91 in which TD-1 and Moomal-2002 were 0.03 genetic distance apart whereas Inqilab-91 was GD = 0.02 from the both genotypes.

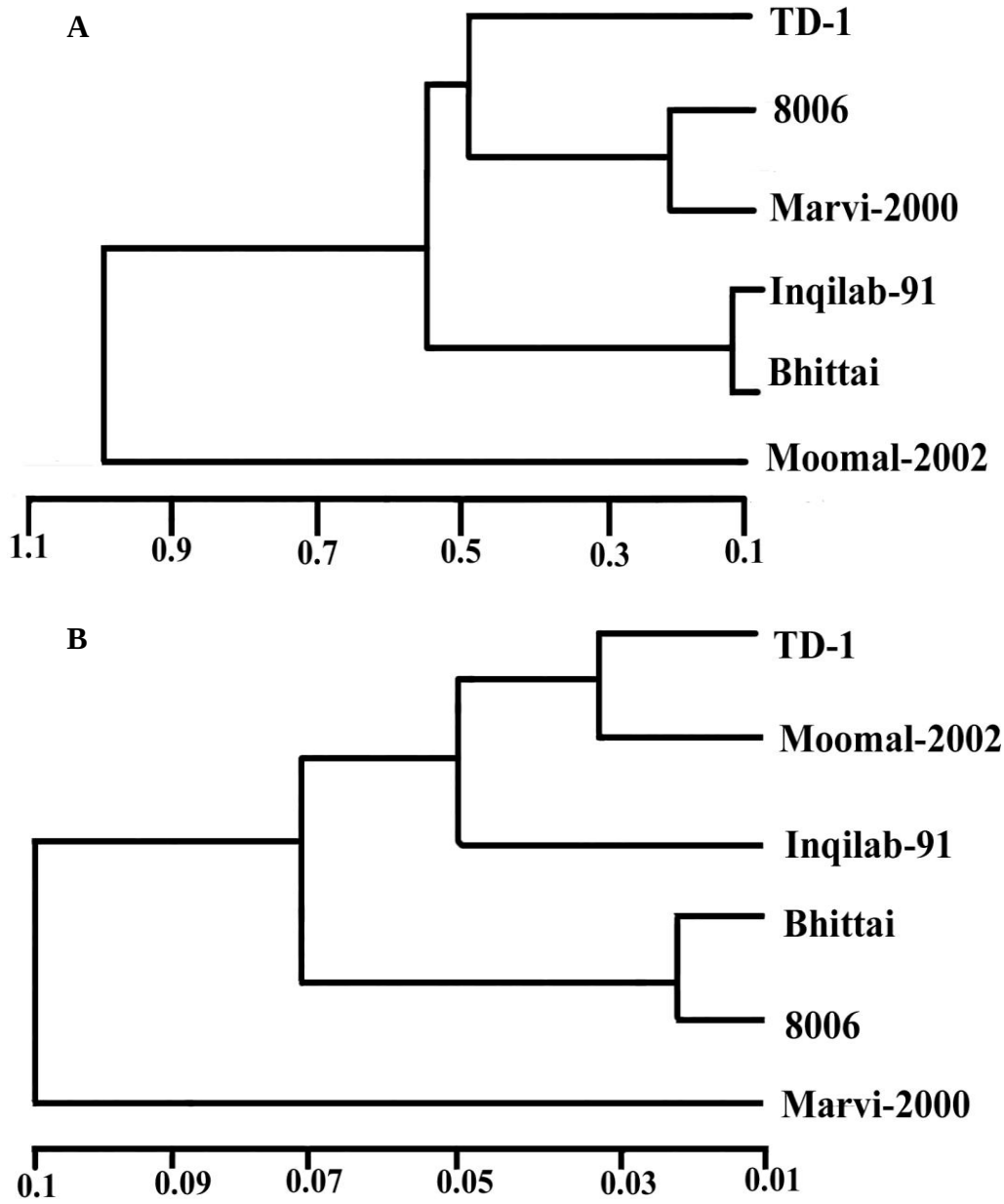

Fig. 2 (A, B). Dendrogram based on Nei (1972), depicting patterns of genetic distance constructed using UPGMA method (A) RAPD (B) SSR markers among six wheat genotypes.

The second group consists of Bhittai and SD-8006, they have maximum similarity in their genetic makeup as they have shown the least GD value i.e., 0.02. The single genotype Marvi-2000 was far apart from the rest of the five genotypes and erected from the hierarchical classification of genetic distance scoring 0.1 GD value.

The correlation coefficient between GS values calculated on RAPD and SSR data was r = 0.52 (significant at 5 % level).

Genetic diversity of loci "h" ranged between 0.04 for OPA-07 to 0.29 for OPB-04 in random marker assay and for SSR it ranged between 0.04 for WMC-124 to 0.185 for WMC-122. Gene diversity is estimated by using Shannon index and the maximum genetic diversity was shown by OPB-04 as compared to the rest of the loci with an average of 0.33 for all loci in RAPD marker study whereas SSR assay unit WMC-122 exhibited the highest value of 0.32 with an average of 0.22 value (Table 7 and 8).

Table 7. Summary of genetic variation statistics for all loci using RAPD markers.

Locus	Sample size	na*	ne*	h*	I*
OPA01	66	2.0000	1.3176	0.2410	0.4053
OPA02	66	2.0000	1.0841	0.0776	0.1694
OPA03	66	2.0000	1.2325	0.1886	0.3368
OPA04	66	2.0000	1.3875	0.2793	0.4524
OPA05	66	2.0000	1.2710	0.2132	0.3696
OPA06	66	2.0000	1.3366	0.2518	0.4188
OPA07	66	2.0000	1.0485	0.0462	0.1120
OPA09	66	2.0000	1.1232	0.1097	0.2221
OPA010	66	2.0000	1.2500	0.2000	0.3521
OPA011	66	2.0000	1.1951	0.1633	0.3018
OPB03	66	2.0000	1.2760	0.2163	0.3736
OPB04	66	2.0000	1.4085	0.2900	0.4652
OPD07	66	2.0000	1.2383	0.1925	0.3420
OPD09	66	2.0000	1.3164	0.2404	0.4044
Mean	66	2.0000	1.2490	0.1936	0.3375
St. Dev.			0.1072	0.0726	0.1043

*na = Observed number of alleles; *ne = Effective number of alleles; *h = Gene diversity (Nei, 1973); *I = Shannon's Information index (Shannon & Weaver, 1949).

Table 8. Summary of genetic variation statistics for all loci using SSR markers.

Locus	Sample size	na*	ne*	h*	I*
WMC-103	120	2.0000	1.0889	0.0817	0.1763
WMC-104	120	2.0000	1.1787	0.1516	0.2852
WMC-105	120	2.0000	1.0897	0.0823	0.1774
WMC-107	120	2.0000	1.0519	0.0494	0.1181
WMC-108	120	2.0000	1.0611	0.0575	0.1335
WMC-109	120	2.0000	1.1087	0.0980	0.2034
WMC-110	120	2.0000	1.1917	0.1609	0.2984
WMC-111	120	2.0000	1.2024	0.1683	0.3089
WMC-113	120	2.0000	1.1400	0.1228	0.2424
WMC-114	120	2.0000	1.2008	0.1672	0.3073
WMC-116	120	2.0000	1.1693	0.1448	0.2753
WMC-117	120	2.0000	1.1603	0.1382	0.2655
WMC-118	120	2.0000	1.2126	0.1753	0.3187
WMC-119	120	2.0000	1.1376	0.1210	0.2397
WMC-120	120	2.0000	1.1693	0.1448	0.2753
WMC-121	120	2.0000	1.2011	0.1675	0.3077
WMC-122	120	2.0000	1.2232	0.1825	0.3285
WMC-123	120	2.0000	1.1705	0.1457	0.2765
WMC-124	120	2.0000	1.0519	0.0494	0.1181
WMC-125	120	2.0000	1.1180	0.1055	0.2155
WMC-126	120	2.0000	1.1687	0.1444	0.2746
WMC-127	120	2.0000	1.1098	0.0989	0.2049
WMC-128	120	2.0000	1.0997	0.0907	0.1914
WMC-129	120	2.0000	1.1282	0.1136	0.2283
WMC-130	120	2.0000	1.0796	0.0737	0.1626
WMC-131	120	2.0000	1.1180	0.1055	0.2155
WMC-132	120	2.0000	1.1376	0.1210	0.2397
WMC-134	120	2.0000	1.0703	0.0657	0.1483
WMC-135	120	2.0000	1.0798	0.0739	0.1630
WMC-136	120	2.0000	1.1089	0.0982	0.2038
WMC-137	120	2.0000	1.1282	0.1136	0.2283
WMC-138	120	2.0000	1.1294	0.1146	0.2297
WMC-139	120	2.0000	1.1477	0.1287	0.2514
WMC-140	120	2.0000	1.1495	0.1301	0.2534
WMC-141	120	2.0000	1.0892	0.0819	0.1767
WMC-142	120	2.0000	1.1486	0.1294	0.2524
WMC-143	120	2.0000	1.2001	0.1668	0.3067
WMC-144	120	2.0000	1.1391	0.1221	0.2413
WMC-145	120	2.0000	1.1605	0.1383	0.2658
WMC-146	120	2.0000	1.1180	0.1055	0.2155
WMC-147	120	2.0000	1.0703	0.0657	0.1483
WMC-148	120	2.0000	1.0613	0.0578	0.1339
WMC-149	120	2.0000	1.1279	0.1134	0.2279
WMC-150	120	2.0000	1.1285	0.0659	0.2286
WMC-151	120	2.0000	1.0705	0.1139	0.1487
WMC-152	120	2.0000	1.1600	0.1380	0.2652
Mean	120	2.0000	1.1317	0.1149	0.2278
St. Dev.		0.0000	0.0465	0.0364	0.0574

* na = Observed number of alleles;* ne = Effective number of alleles;* h = Gene diversity (Nei, 1973);* I = Shannon's Information index (Shannon & Weaver, 1949).

Discussion

Comparative studies of RAPD and SSR markers have been done in a wide range of crop species including corn (Smith & Helentejaris, 1998), soybean (Powell *et al*., 1996; Brown-Guedira *et al*., 2002), barley (Russell *et al*., 1997), sorghum (Agrama & Tuinstra, 2003; Uptmoor *et al*., 2003), rice (Fugang *et al*., 2003) and wheat (Jones *et al*., 1997).

In RAPD analysis, a large number of bands were revealed due to the random priming nature and potential confounding effects associated with co-migration with other markers (Tessier *et al*., 1999). Several studies compared various molecular marker systems in plants and the SSR was highly correlated with the morphological markers in contrast to AFLP and RFLP (Geleta & Labuschagne, 2005). Panwar *et al*., (2010) compared the efficiency and effectiveness of RAPD and the SSR markers in finger millet. Similar studies for comparison of marker systems were also reported by Agrama & Tuinstra (2003). The earlier findings have generally revealed a good congruence between the genetic patterns gained by the two genetic marker systems. In the present finding, RAPD and SSR allowed to distinguish wheat genotypes and to reveal genetic diversity among the six major cultivars of bread wheat (CCN resistant) used in Pakistan. A high genetic diversity was found between Moomal-2002 and SD-8006 on the basis of the RAPD data at GD=1.02 value but when the same genotypes were screened for their diversity by SSR markers it has been evaluated that they were existed much closer having GD value 0.032. Marvi-2000 is the only genotype diversified genetically from the remaining five cultivars after being screened by microsatellites. In the RAPD analysis, the genetic distances ranged from 0.1 to 1.0. The results concur with Grenier *et al*., (2000) who reported diversity range of 0.71 to 0.93. The RAPD marker analysis showed some distinction among the genotypes and most of them were very similar. Ayana *et al*., (2000) also reported weak differentiation of Ethiopian and Eritrean sorghum accessions after using RAPD markers.

On the other hand, the SSR analysis showed genetic similarity values ranging from 0.02 to 0.1. When SSR and RAPD analysis were compared, similarity matrices constructed based on shared allele analysis revealed the lowest average genetic similarity between genotypes when estimated using SSR markers (0.33) and was higher among entries when determined using RAPD markers (0.62). These results indicate that RAPD markers provide less resolving power than SSR markers. RAPD markers have screened the diversity in the broad spectrum whereas microsatellite can detect the diversity with high resolution capability, therefore, they can easily rule out RAPD utility. Microsatellite primer pairs are single locus markers where as RAPD markers are not considered much reliable than SSR due to their multi-locus nature. Since only specific locus was expected to be amplified by each SSR primer (Gupta *et al*., 1999); secondly, the microsatellite loci are also multi-allelic (Prasad *et al*., 2000) therefore, these qualities make SSRs superior in detecting DNA polymorphism and useful alternate molecular marker.

Navaz *et al*., (2009) reported that the highest percentage of polymorphism was observed using RAPD primers from 25 to 18.75% for four genotypes. The level of polymorphism was 16% more using SSR as compared to RAPD markers whereas average number of polymorphic loci was 44.39/genotype and 13.21/ genotype, respectively. Therefore, SSR marker work effectively to reveal polymorphism and can be used as criteria for practical breeding.

The estimates of the genetic similarity (GS) coefficients between the pairs of genotypes, ranged between 0.687 to 0.996 and 0.798 to 0.999 for RAPD and SSR, respectively. The average value of GD was 0.93 for RAPD and 0.88 for SSR suggesting that the six genotypes used in the study were diverse. This value can be compared with those reported in earlier studies, where SSR based coefficient value was 0.31 (Plaschke *et al*., 1995), 0.57 (Bohn *et al*., 1999), 0.80 (Masmoud *et al*., 2006), 0.40 and 0.12 for RAPD and SSR, respectively (Naghavi *et al*., 2004).

It is considered that the variation in GS coefficient values may be attributed either in the number of genotypes and the primers used.

Semegan (2002) defined two reasons for showing low correlation between molecular markers and agronomic traits. First, molecular markers act on more percentage of genomes than morphological traits such as intron and exon regions. Secondly, there is no exposure of artificial selections.

The RAPD and SSR assay reflect restriction size variation spread across the genome, because the use of RAPD markers resulted in the greatest average number of alleles per locus as compared to the other marker systems tested. We found that estimates of polymorphism information content (PIC) based on RAPD 23% and SSR 78% measures had the lowest standard deviations and were the most informative. Although the RAPDs and SSRs markers gave the lowest mean PIC value, they provided a similar degree of PIC as by Khavarinejad & Karimov (2012).

Recommendation for CCN resistant genotypes can be effectively made after sum up the entire genotypic and phenotypic capabilities of a genotype that has unique edge in resistance against prevailing virulence of different diseases. In the present study, six wheat genotypes that are the leading varieties of the country are graded on the basis of genetic diversity that also possess distinct morphological characteristics which make them different from other existing cultivars of Pakistan.

One of the variety, Bhittai is capable to resist against stem, leaf, stripe rust but susceptible to yellow rust that is inflexible in Sindh (Anon, 2009). SD-8006 produce high average yield 5818 kg/ ha in irrigated areas of Punjab but susceptible to stem and leaf-rust.

The variety Marvi-2000 exhibited highest yield among these six genotypes. It has potential against most of the wheat diseases and maintained its superiority over local check varieties at various agro-ecoclimatic zones, which confirmed its wide adaptability. Hence the deployment of Marvi-2000 will enhance sustainable wheat production.

Marvi-2000, found to be both resistant to CCN and genetically most diverse. It is recommended to end-users. Due to availability of genetic variability information that is pre- requisite for any breeding program aimed towards the improvement of wheat productivity.

RAPD and SSR markers ensured the valid differentiation and the selection of bread wheat varieties based on the polymorphic rates. Maximum primer combinations provide much authentic results for genetic diversity assessment. Due to hyper variability and high-resolution capability, SSR is more effective tool to reveal diversity than RAPD and can be used as criteria for practical breeding.

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